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PRINCIPAL INVESTIGATOR: Sai-Ching Jim Yeung

CONTRACTING ORGANIZATION: The University of Texas M. D. Anderson Cancer Center
1515 Holcombe Blvd
Houston, TX 77030

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Sai-Ching Jim Yeung

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14. ABSTRACT

Epidemiological studies have identified that type 2 diabetes mellitus (DM2) is a significant risk factor for carcinogenesis and cancer death. A few recent studies have also shown that different antidiabetic treatments have different impact on cancer. This proposal is to address the impact of antidiabetic treatments on the survival of breast cancer. We participated in the Era of Hope meeting in Chicago and presented our cell culture data in a poster session. We have successfully bred the mouse model of HER2-positive breast cancer and diabetes mellitus, i.e., MMTV-ErbB2 (neu): db/db. Based on the mice with 50% FVB and 50% C57BL6 background that we currently observed, MMTV-neu: db/db are developing breast cancer earlier and surviving shorter than their lean litter mates. Experiments are in progress to examine angiogenesis, cell proliferation and apoptosis in the mouse breast tumors. Breeding is in progress to produce animals for the next phase of the animal study to examine the impact of antidiabetic treatments on the survival of these diabetic mice with breast cancer.

15. SUBJECT TERMS
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The Impact of Diabetes Treatment on survival in a Breast Cancer/Diabetes Model

Interim Research Report/Body:

Introduction

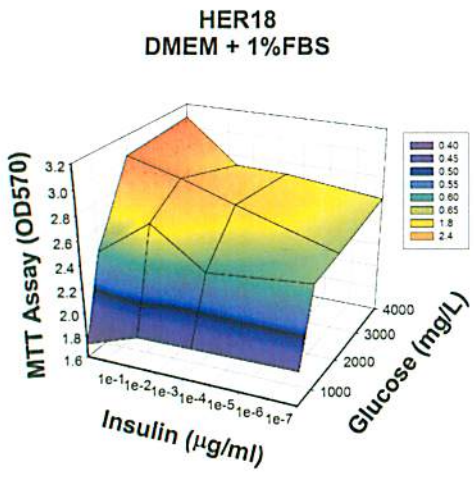
Epidemiological studies have identified that type 2 diabetes mellitus (DM2) is a significant risk factor for carcinogenesis and cancer death. A few recent studies have also shown that different antidiabetic treatments have different impact on cancer. This proposal is to address the impact of antidiabetic treatments on the survival of breast cancer. In Specific Aim 1, we shall determine the impact of DM2 on the angiogenesis, proliferation, apoptosis and survival of breast cancer in a mouse model of DM2 and breast cancer. In Specific Aim 2, we shall determine if different treatments of diabetes differentially improve survival in mice with breast cancer and DM2.

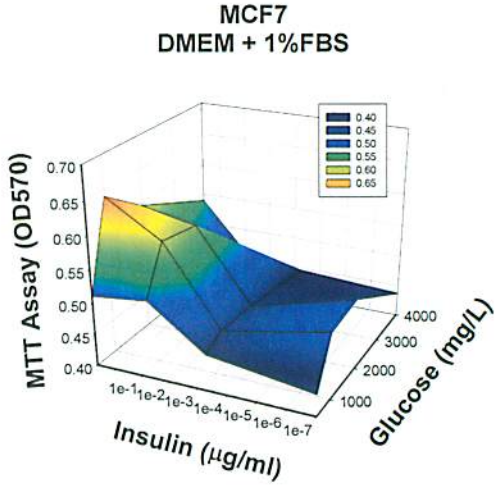
Key Research Accomplishments

1. We have demonstrated in cell culture that insulin and glucose promoted breast cancer growth while metformin, rosiglitazone and orlistat inhibited breast cancer growth and induced apoptosis. Exenatide did not have any significant direct impact on the breast cancer cells. These in vitro data imply that the choice of pharmacotherapy for DM2 in breast cancer patients will influence the oncologic outcome of these patients. Parts of these data were presented at the Era of Hope Conference in Chicago in 2008.
2. We have successfully generated a mouse model of HER2-positive breast cancer and diabetes (MMTV-ErbB2/ lepr $^{db/db}$)by breeding MMTV-ErbB2 transgenic mice with mice that carry the lepr ^{db} mutation. MMTV-ErbB2/ lepr $^{db/db}$ mice are obese and diabetic, and their cancer specific survival is significantly shorter ($P<0.05$) shorter than their lean littermates. These data provide strong in vivo evidence that diabetes shortens cancer survival. Work is in progress to characterize the tumor samples from these mice as well as the hormonal and metabolic characteristics of these mice. Breeding is also in progress to generate more mice of this model to test the differential impact of antidiabetic drugs on breast cancer survival

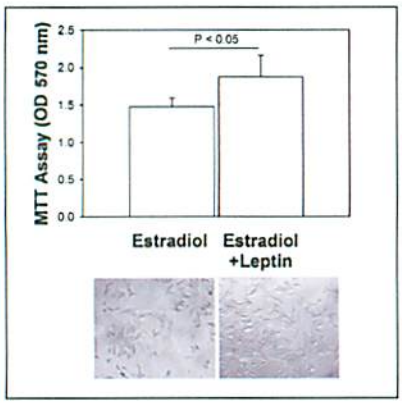
Reportable Outcomes

Diabetes mellitus type 2 (DM2) is well-known to be associated with increased risk as well as adverse outcome of breast cancer. Despite early investigations into mechanisms linking DM2 and breast cancer, only limited studies focused on translating this knowledge into potential clinical interventions that could modify the risk or outcome of diabetic breast cancer patients.



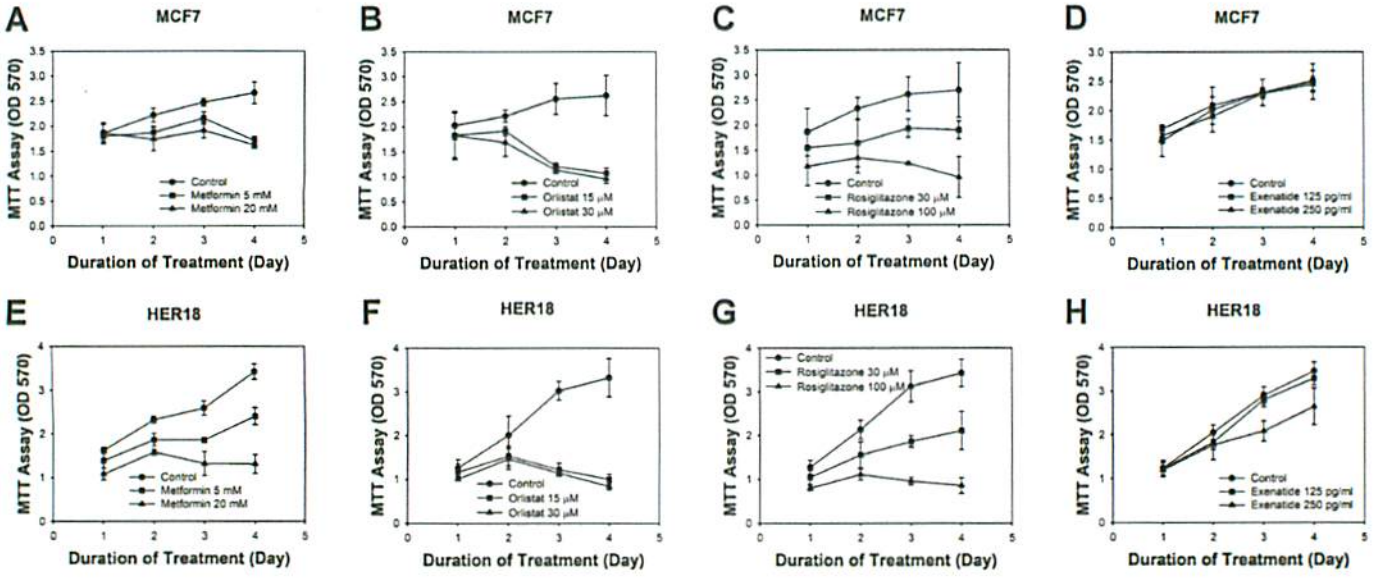


Insulin and glucose promote the proliferation of breast cancer cells, but the growth promoting effect of glucose was dependent on insulin and was observed only at high insulin concentrations. Beginning with the same number of cells, the number of live cells were examined by the MTT assay after culturing for 3 days in DMEM without glucose + 1%FBS with glucose and insulin added at various concentrations. The above 3-D surfaces demonstrated the relative dominance of insulin over glucose on the proliferation of MCF7 cells and the relative dominance of glucose over insulin on the proliferation of HER18 cells. Therefore, antidiabetic treatments that increase insulin may stimulate breast cancer proliferation.

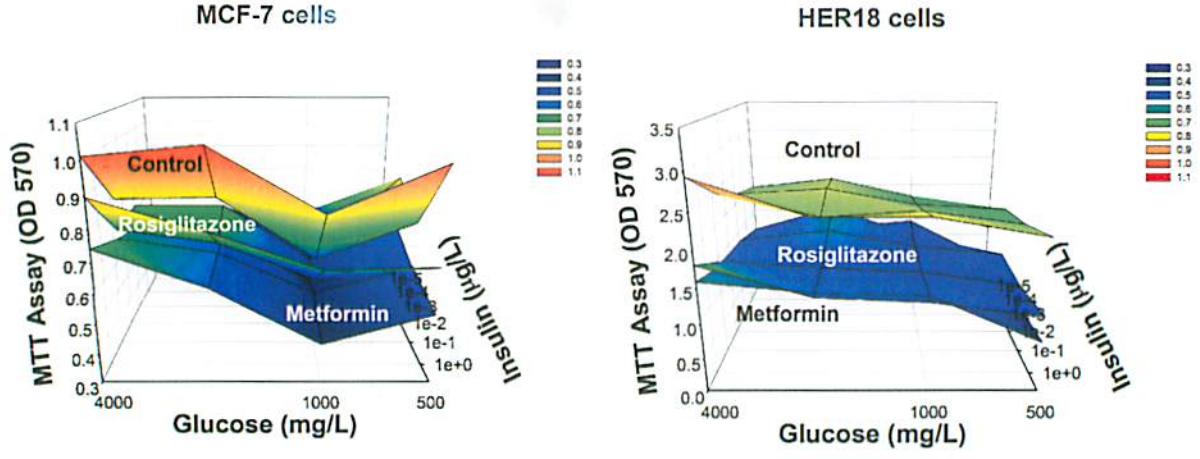


Many patients with type 2 diabetes are obese, obese females have higher leptin and estrogen levels. Leptin and estrogen stimulate breast cancer cell growth. The respective cultures are shown at the same magnification below. Note that number of cells treated with both leptin and estradiol is more than that of cells treated with estradiol alone.

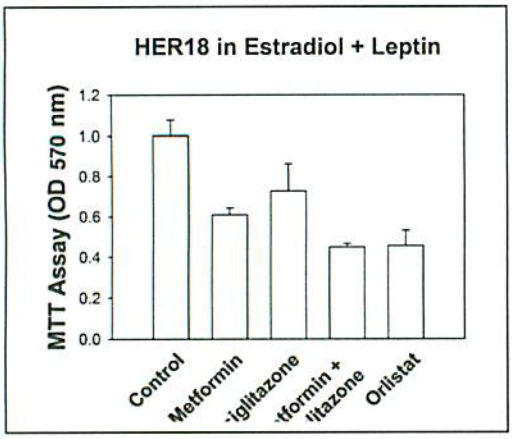
Several other classes of antidiabetic treatments that do not increase insulin were examined. Metformin, a biguanide, activates AMPK, and rosiglitazone, a PPAR-γ agonist, increases the protein level of PTEN. Direct anticancer effects of metformin and rosiglitazone have not been examined in the context of varying degrees of hyperinsulinemia and hyperglycemia. Exenatide, an incretin analog, has no known antineoplastic effects. Orlistat, an antiobesity drug that blocks fat absorption, is also a fatty acid synthase inhibitor.



Breast cancer cells MCF or Her18 were treated with indicated amount of drugs. Metformin, rosiglitazone and orlistat inhibited proliferation of cancer cells while exenatide had no effect on MCF7 and a small effect on HER18.

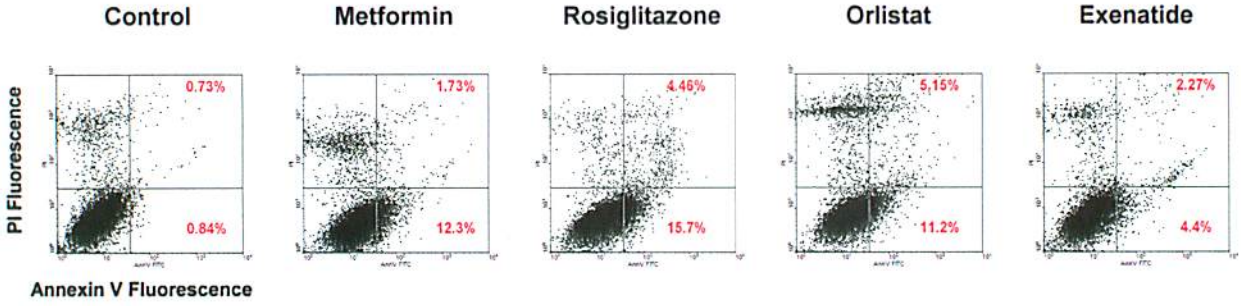


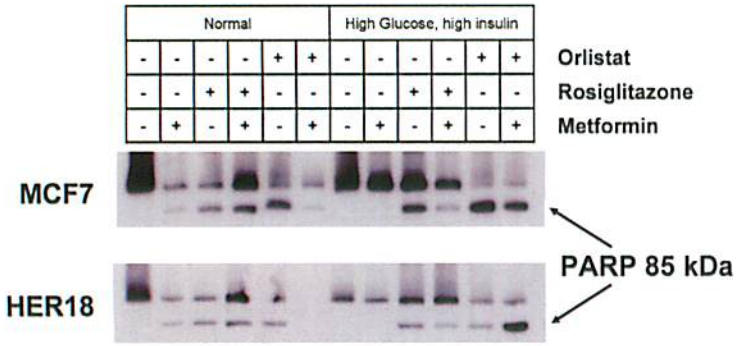
The above 3-D surfaces demonstrated that the inhibitory effects of rosiglitazone and metformin on MCF7 and HER18 cells were present at all the insulin and glucose concentrations tested.



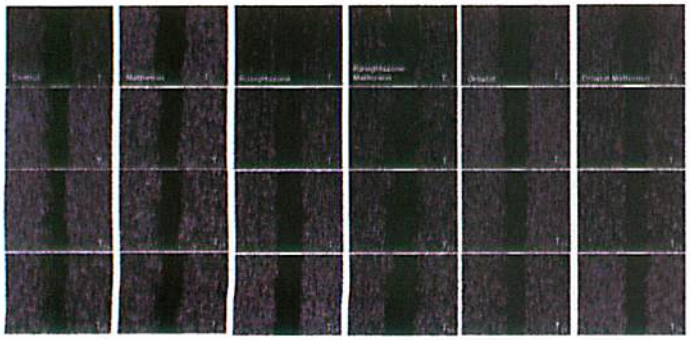
HER18 cells cultured in leptin and estradiol were treated with indicated drugs for 72 hours followed by MTT assay for analyzing growth inhibition.

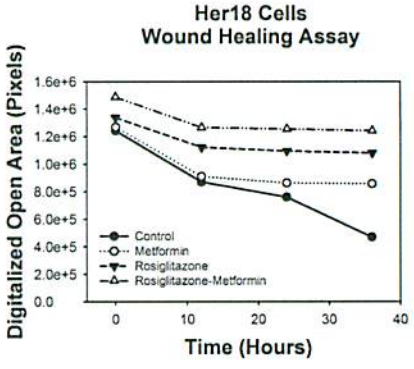
Insulin-sensitizing or anti-obesity drugs can cause apoptosis in breast cancer cells. The next figure shows flow cytometry analysis of breast cancer cell HER18 cells treated with the indicated drugs. Cells were treated with the indicated drugs (metformin 5 mM, rosiglitazone 100 μM, orlistat 15 μM, and exenatide 125 pg/ml) for 48 hours and then stained with propidium iodide and annexin V-FITC followed by flow cytometry. The percentages of annexin V positive (apoptotic) cells are indicated. Similar results were obtained with MCF7 cells.



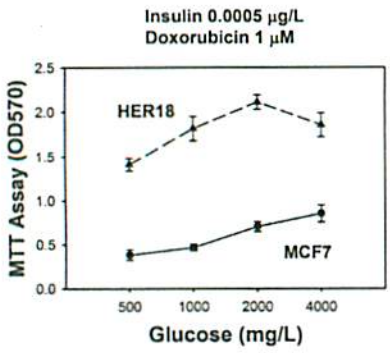


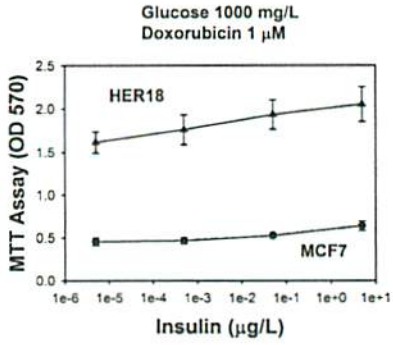
The induction of apoptosis by metformin, rosiglitazone and orlistat in MCF7 and HER18 cells was also confirmed by specific cleavage of PARP. The immunoblot on the left showed the 85 kDa fragment of PARP (arrows).



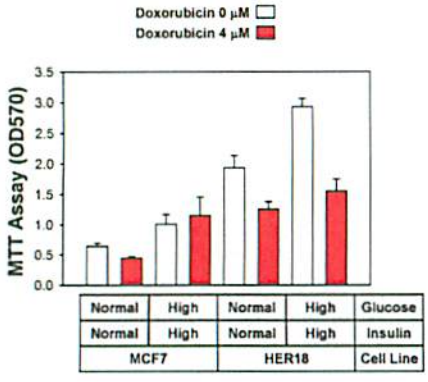


The impact of metformin, rosiglitazone and orlistat on breast cancer cell motility was evaluated using the scratch wound healing assay in cell culture. The area of the empty surface on the tissue culture plate was measured in pixels using the Image J software. The data for HER18 cells were plotted over time. Rosiglitazone and metformin inhibited HER18 cell motility.





Both insulin and glucose contributed to the chemoresistance of breast cancer cells to doxorubicin. The number of live cells as represented by the optical density (OD) in the MTT assay is plotted against glucose (left above) or insulin (right above). All the cells were cultured in the presence of 1 µM doxorubicin. All the error bars represent 95% confidence intervals.



The combination of high glucose (4000 mg/L) and high insulin (5 µg/L) increased the number of live cells in both MCF7 and HER18 cells in the presence of 4 µM doxorubicin. For MCF7 cells in the presence of high insulin and glucose, the inhibitory effect of doxorubicin disappeared.

In summary, our results showed that culture conditions mimicking hyperinsulinemia and hyperglycemia stimulated breast cancer cell proliferation and conferred chemoresistance to doxorubicin. Two antidiabetic drugs commonly used for DM2, metformin and rosiglitazone, had direct antineoplastic effects, and so did an anti-obesity drug, orlistat. These results have significant therapeutic implications in terms of the choice of pharmacotherapy for DM2 in the context of diabetic breast cancer patients.

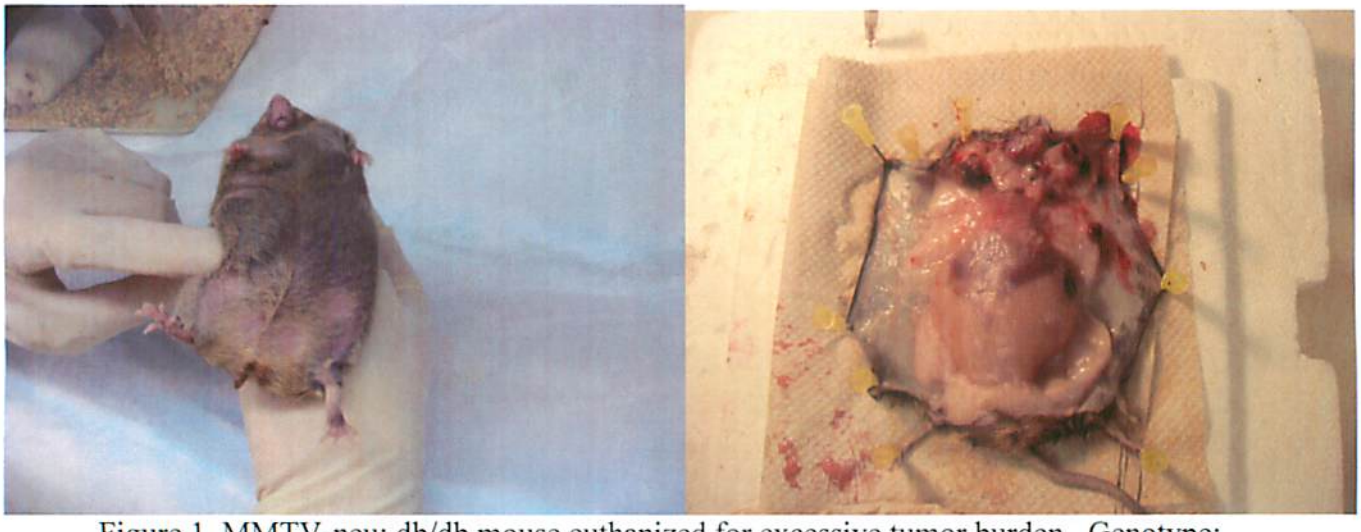
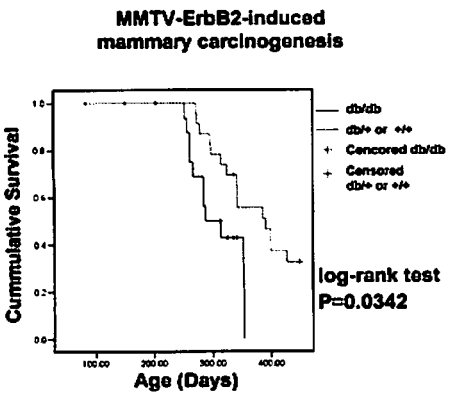
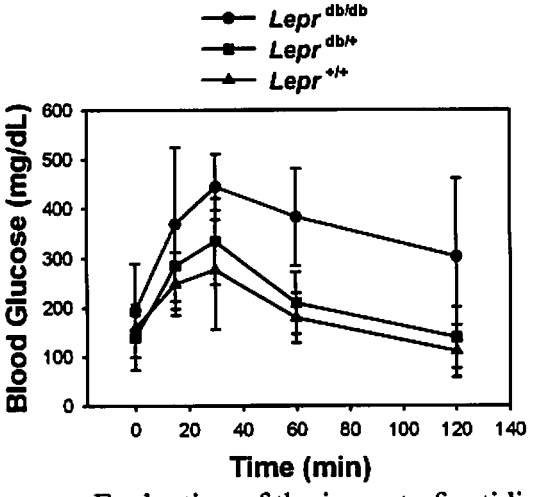


Figure 1. MMTV-neu: db/db mouse euthanized for excessive tumor burden. Genotype: Tg(MMTV-Erbb2)NK1Mul; $Lepr^{db}$ $Lepr^{db}$
Body weight: 68 g. Note the breast carcinomas in the upper part of the body and the extensive white fat pad in the lower part of the body.



We have successfully developed an animal model of breast cancer and diabetes. Obese diabetic mice db/db are bred with transgenic mice MMTV-ErbB2 (neu) to generate mice that carry the MMTV-neu transgene and are homozygous for db mutation. These mice are obese and diabetic and they develop mammary cancers. A small percentage of mice have morbid obesity impairing the ability to obtain food and water and were euthanized as required by the animal protocol prior to cancer development, and these animals are censored from the survival analysis. The tumor-specific survival of these diabetic mice was compared with non-diabetic lean littermates, and the db/db mice have significantly shortened tumor-specific survival (log rank test, P=0.0342).



Glucose tolerance tests were performed in the mice prior to euthanasia for excessive tumor burden. The chart to the left shows significant elevation of glucose in the diabetic db/db genotype. The error bars represent 95% confidence intervals.

Evaluation of the impact of antidiabetic treatments on the breast cancer and survival of these animals will begin as soon as enough animal are bred. Large scale breeding to obtain the mice of the appropriate genotype and sex is in progress. We are confident that our hypothesis is correct that different antidiabetic treatments have different impact on breast cancer in diabetic subjects. Our experiments will provide strong in vivo evidence to justify future clinical trials.

Conclusion

The project is progressing well. We have generated strong data demonstrating that diabetes shortens breast cancer survival, and there is very likely to be differential impact of antidiabetic medications on the survival of breast cancer. We are in the process of carrying out experiments to prove that different antidiabetic medications do have different impact on breast cancer survival in vivo using the animal model that we have successfully generated.

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Office of Research Administration
Unit 176
phone 713-563-3888
Fax 713-794-4535

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04/30/2007

To: Sai-Ching J. Yeung/MDACC
Copy: Rosie M. Handy/MDACC

From: Lydia G. Jackson/MDACC

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Version: 01
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PROTOCOL STATUS: APPROVED AND ACTIVATED

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Meeting Date: April 17, 2007
Approval Date: April 30, 2007
Expiration Date: April 2010

Number of animals approved: 885

The IACUC has reviewed and approved the above animal protocol. The Office of Research Administration has activated the protocol for use. For information concerning this protocol, please contact your appropriate IACUC Coordinator.

Sincerely,

Lydia G. Jackson
04/30/2007 05:30:05 PM